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Short Communications

The effects of knife cuts of hippocampal pathways on epileptic activity in the seizure-sensitive gerbil

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Previous studies have shown morphological differences in the hippocampal formation of seizure-sensitive gerbils as compared to seizure-resistant gerbils. To determine the significance of these differences, lesions were made of hippocampal afferents and efferents. Seizure-sensitive gerbils with bilateral knife cuts of the perforant path, including those with bilateral fornix lesions, showed no seizure activity following surgery. However, bilateral transections of the fimbria of the fornix, unilateral lesions of the perforant path and sham surgeries had no significant effect on seizure activity. The termination of seizure activity with bilateral lesions of the perforant path suggests that this pathway, as opposed to the fornix, is required for motor seizures in this strain of gerbils.

The Mongolian gerbil provides a good model for the study of genetic epilepsy because the animals offer good genetic control, breeding proclivity and ease of behavioral testing[4]. The gerbils exhibit spontaneous motor seizures in response to a variety of stimuli[20]. For example, the novelty of moving them from one cage to another is adequate to develop a seizure. The seizures exhibited by the gerbils are consistent over time and, therefore, it is possible to correlate seizure intensity records with morphological observations.

Previous studies from this lab have shown a specific defect in the GABAergic system in models of focal epilepsy[12–16]. Basically, GABAergic neurons are preferentially lost at epileptic foci in the neocortex and these results are consistent with physiological and pharmacological data[6,11]. In contrast, the hippocampus of the seizure-sensitive (SS) gerbil displays a paradoxical increase in the number of GABAergic basket cells in the dentate gyrus[10]. This increase in GABAergic neurons is also associated with an increase in GABAergic terminals and a paradoxical change in the morphology of granule cell axon terminals in that they have features indicating high activity[10].

Other morphological studies of the SS gerbil suggest that the hippocampus is a specific brain site for seizure-related changes. Paul et al.[8] have shown a reduction in the density of spines on dendrites of pyramidal cells in the CA_3 region of the SS hippocampus. Also, other immunocytochemical data from our laboratory indicate that the increased number of GABAergic neurons appears to be specific for the SS hippocampus because other structures in SS brains, such as the motor cortex, substantia nigra and thalamic reticular nucleus do not display any differences from that found in seizure-resistant gerbils[9]. Although significant losses of both Purkinje cells in the cerebellum and pyramidal cells in the hippocampus were reported for SS gerbils, these losses were considered to be a result of the seizure activity[2,7]. In contrast, the increased number of GABAergic basket cells in the SS dentate gyrus is more likely involved with the cause of seizure activity because it occurs in young

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progeny of SS gerbils prior to the onset of seizure activity[10]. The following study was initiated to determine if surgical isolation of the hippocampal formation from its connections with other brain regions would inhibit seizure activity in the SS gerbil.

This study utilized progeny of gerbils obtained from Dr. Arnold B. Scheibel's laboratory at the University of California, Los Angeles. Spontaneous seizures were exhibited by adult SS gerbils when they were introduced to a novel environment. Seizure intensities were graded in a series of 9 steps, from +1 to +5 with increments of 0.5. On this scale, a 0 indicated seizure absence. Briefly, a +1 designated a twitching of vibrissae and ears, and a +2 involved similar movements as well as having the head bob up and down. The more severe +3 rating was used when the vibrissae and ears twitched rapidly, the head stayed flattened against the floor and the body looked humped in a tense little mound. The most severe ratings (+4 and +5) are characterized by extreme body flattening with lordosis, repetitive pawing of the ground, tonic–clonic movements of the limbs, and wild running and leaping into the air, which end in rolling over in seizures of +5. Gerbils from both strains were tested once a week individually as described by Paul et al.[8]. The 16 gerbils which survived the surgery had a mean number of 12 tests (range 4–32) performed prior to the time of surgery. When tested, the gerbil was removed from its home cage and placed in a large empty stainless-steel container where it was observed for 5 min. The seizure intensity or an absence of seizure was determined and recorded once a week. Concurrently, an unoperated group of gerbils were tested as a control. Their seizure records remained consistent over this time period.

Five types of stereotaxic surgeries were performed on SS gerbils with consistent seizure records: (1) bilateral lesions of the perforant path from the entorhinal cortex, (2) unilateral lesions of the perforant path, (3) bilateral lesions of the fimbria of the fornix, (4) bilateral lesions of both the perforant path and the fornix (see Fig. 1), and (5) sham surgeries with only the cranium opened bilaterally at two sites. The gerbils were anesthetized with Nembutal. The hair on the dorsum of the head was shaved and the head of the animal was placed in a stereotaxic instrument. The coordinates for the perforant path and/or the

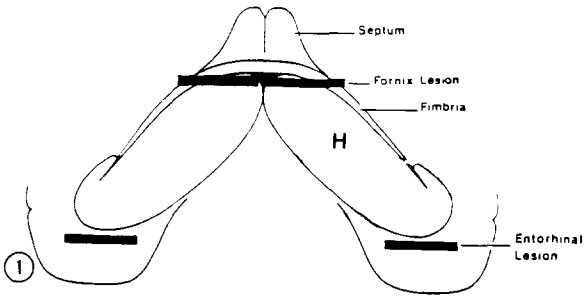

Fig. 1. Schematic drawing of a dorsal view of the hippocampal formation (H) and associated structures to indicate the locations and extent of fornix and perforant path lesions.

fornix were then determined from a stereotaxic atlas of the gerbil brain[5]. The perforant path coordinates were determined to be −5.0 mm dorsoventrally (D-V), −3.2 rostrocaudally (R-C) relative to bregma and ±4.0 laterally from the midline (M-L). The fornix lesion coordinates were determined to be −2.0 mm D-V at −0.8 mm R-C and ±1.5 M-L. A midline skin incision was made and a drill was used to penetrate through the cranium at the selected points determined from the stereotaxic atlas. Then,lesions of the perforant path fibers from the entorhinal cortex and/or the fibers of the fornix were made with a sterile surgical scalpel blade (Bard-Parker, no. 11). The blade is not wider than 0.5 mm at its tip and is approximately 2 mm wide at a distance 5 mm from the tip. The surgical blade was oriented perpendicular to the surface of the brain and passed down to the appropriate depths (either 2 mm ventral to the cortical surface for the fornix lesions or 5 mm ventral to the cortical surface for the perforant path). The knife blade was moved in the coronal plane a few millimeters back and forth to insure the disruption of brain tissue. After the surgery, the incisions were closed and the animals tested once a week for 5 weeks. Since the gerbils were coded, there was no knowledge of the lesion(s) for each animal to insure equal treatment during the testing.

After the postoperative testing period, anesthetized gerbils were perfused transcardially with 0.9% saline solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at 4 °C. The brains were dehydrated in a series of alcohol and embedded in paraffin. Horizontal brain sections were cut from these paraffin-embedded blocks on a rotary microtome at 10 μm thickness. Every tenth section was

mounted on glass slides and stained with the Nissl method. Slides were examined with a light microscope to determine the location and extent of the lesions. Then, the code was broken to assign seizure records for the various types of lesion.

The bilateral lesions of the perforant path and/or the fornix as well as the unilateral lesions of the perforant path were confirmed in the histological preparations (Figs. 2 and 3). The lesions of the perforant path created significant damage to the occipital cortex overlying the hippocampal formation. More ventrally, increased gliosis and large spaces were found in the presubiculum and subiculum with presumed interruption of the perforant path fibers from the entorhinal cortex. Shrinkage of the molecular layer of the dentate gyrus (Fig. 3) confirmed this assumption because this site is a major termination of perforant path fibers. Occasionally, the lesion extended to include the temporal pole of the dentate gyrus and hippocampus. The lesions of the fornix caused less trauma to overlying cortex because the knife blade was lowered to only 2 mm below the cortical surface. The fornix displayed a reduction in its size as well as gliosis at the site of transection (Fig. 2).

The initial experiments showed that gerbils with bilateral lesions of both the perforant path and fornix displayed no seizure activity following the surgery (see Table I). Prior to stereotaxic surgery, the 3 gerbils in this group had an average seizure rating of 3.2. A second group of 5 gerbils had bilateral perforant path lesions and they showed no seizure activity after the surgery. This latter group had a somewhat lower presurgical seizure rating ($\bar{x} = 2.5$). A group of 4 gerbils had bilateral lesions of the fornix. These animals had average seizure ratings of 1.7 prior to surgery and 2.6 following surgery (Table I). One of the 4 gerbils in this group did not have any seizures following the surgery. However, it is interesting to note that this latter gerbil had a low seizure rating (1.6) prior to surgery. In contrast, the other gerbils with fornix le-

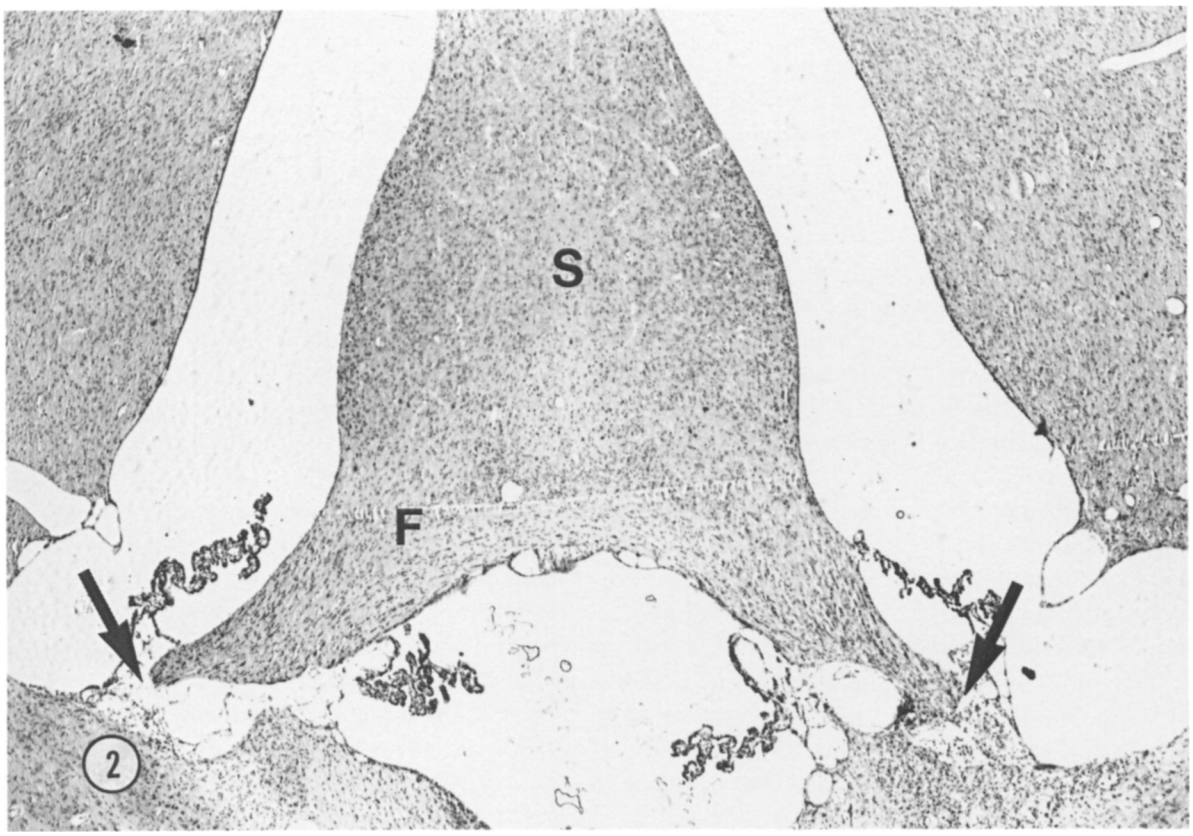

Fig. 2. Photomicrograph of a horizontal section through the fornix (F) and septum (S) to indicate the bilateral lesion sites (arrows) ×60.

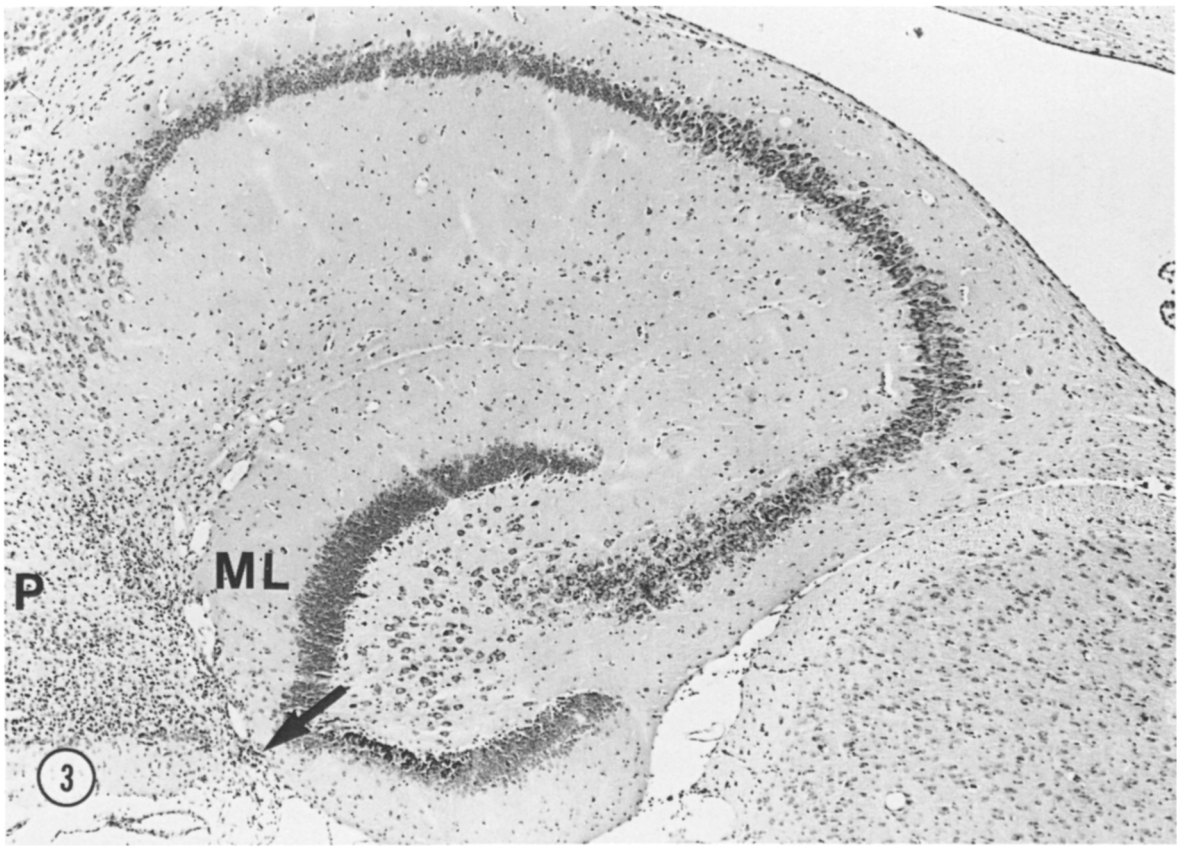

Fig. 3. Photomicrograph of a horizontal section through the temporal pole of the hippocampus and part of the presubiculum (P). The site of the lesion (arrow) displays increased numbers of glial cells. Also, the molecular layer (ML) of the dentate gyrus has shrunken at this site indicating a loss of many afferent axons from the entorhinal cortex. ×50.

sions showed an increase in epileptic activity (Table I).

To determine if the perforant path effect of blocking seizures is bilateral, two additional gerbils with unilateral lesions of the perforant path were evaluated. They continued to exhibit seizure activity after the surgery (Table I). No effect on seizure activity was also observed for the two sham-operated gerbils (Table I). Non-parametric statistical tests were used to show that differences existed between groups (Kruskal–Wallis, df 9, $H = 18.5$, $P < 0.05$). The Wilcoxon's signed rank test showed that only the bilateral perforant path transections and those combined with fornix transections displayed significant differences ($P < 0.05$).

These findings are interesting in light of our previous studies that showed a significant increase in the number of GABAergic basket cell neurons in the septal pole of the hippocampal dentate gyrus of gerbils and a significant increase in the number of GABAergic neurons in the apical dendritic field of CA_3 without a significant difference in the number of GABAergic neurons in other brain regions[9,10]. The fact that seizures were eliminated in SS gerbils with bilateral destruction of the perforant path suggests that the hippocampal formation has an abnormal circuitry that might be involved in the generation and/or propagation of epileptic activity. This finding is consistent with the significant bilateral increase in GABAergic neurons that occurs in the rostral part of this structure[10]. In corroboration with this bilateral defect is the fact that unilateral lesions of the perforant path maintained or may have slightly exacerbated seizure activity in SS gerbils. The finding that lesions of the input from the fornix to the hippocampus had no significant effect on seizure activity suggests that the perforant path input has a stronger effect on the generation and/or propagation of seizure activity in the hippocampal formation. This should be

TABLE I

Effects of knife cuts of hippocampal pathways on seizure behavior in seizure-sensitive gerbils

A, bilateral fornix and perforant path transections; B, bilateral perforant path transections; C, bilateral fornix path transections; D, unilateral perforant path transections; E, sham-operated gerbils.

	Gerbil	*Age*	*Sex*	*Seizure score*	
				Before surgery	*After surgery*
A	80	8 months	F	4.8	0
	28	2 years	M	2.7	0
	68	8 months	M	2.0	0
				$\bar{x} = 3.2$	$\bar{x} = 0$
B	56	8 months	M	4.1	0
	55	8 months	M	3.5	0
	49	17 months	M	2.1	0
	58	8 months	M	1.9	0
	82	8 months	F	1.0	0
				$\bar{x} = 2.5$	$\bar{x} = 0$
C	52	17 months	M	3.1	3.8
	50	17 months	M	1.6	0
	67	8 months	M	1.2	3.4
	90	8 months	M	1.0	3.3
				$\bar{x} = 1.7$	$\bar{x} = 2.6$
D	77	8 months	F	1.6	2.3
	92	8 months	F	3.8	4.3
				$\bar{x} = 2.7$	$\bar{x} = 3.3$
E	50a	8 months	M	4.6	4.75
	66	8 months	M	1.5	1.75
				$\bar{x} = 3.1$	$\bar{x} = 3.3$

expected because the perforant path contains a larger number of afferent fibers than the fornix.

The size of the lesions were often larger than the perforant path tract. Therefore, this effect may be due to the inclusion of other fiber systems that may connect the hippocampal formation to the entorhinal cortex and subiculum. Since attempts to destroy the granule cells of the dentate gyrus with colchicine failed (personal observation), knife cuts were the next best alternative for creating a discrete lesion.

Lesion studies have provided valuable information for other models of epilepsy. For example, bilateral lesions of the inferior colliculus block audiogenic seizures in the genetically epilepsy prone rat whereas lesions of more rostral auditory structures such as the medial geniculate body and auditory cortex have no effect[3,21]. It is interesting to note that the inferior colliculus of this rat displays a significant bilateral increase in the number of GABAergic neurons[17]. Thus, the abnormal GABAergic circuitry in the inferior colliculus may contribute to the propagation or generation of seizure activity in this structure which apparently mediates its final motor outflow via the midbrain and pontine reticular formation[1]. In addition, and more pertinent to the present findings, Savage et al.[18] have shown the importance of an intact perforant path for another model of epilepsy, amygdaloid kindling. The fact that perforant path lesions can block other types of seizure activity may indicate the critical role for this pathway in the propagation of a variety of seizures that originate in the forebrain.

The seizures in SS gerbils are provoked by stress or changed environmental cues. These polymodal stimuli probably enter the hippocampus via the perforant path because many cortical association areas have

projections to the entorhinal cortex[19]. It is possible that the hippocampus of SS gerbils might be amplifying this cortical activity as it funnels in from the entorhinal cortex.

The increased activity that may occur in the hippocampus of SS gerbils must spread to other regions of the cortex to ultimately have an effect on the motor cortex or some other structure with major descending spinal cord projections. Since fornix lesions alone had no significant effect on seizure activity, the output of the hippocampus along this pathway is probably not involved with a major projection to a motor structure. Another possibility is that the hippocampal output to a motor brain region travels via the perforant path back to the entorhinal cortex because lesions of the perforant path terminate seizure activity in SS gerbils. This alternative is likely because a reciprocal projection exists between the hippocampus and the entorhinal cortex[19] and this latter region may ultimately project to motor structures.

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